MICROSTIMULATION OF LUMBOSACRAL SPINAL CORD-MAPPING

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Ninth Progress Report
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THIS QPR IS BEING SENT TO YOU BEFORE IT HAS BEEN REVIEWED BY THE STAFF OF THE NEURAL PROSTHESIS PROGRAM.

I. Introduction

During this quarter we continued work in three areas: (1) the examination of sites in the lumbar cord which produced flexion and extension about the knee joint to spinal cord microstimulation. In these studies we addressed two issues which have been mentioned in previous progress reports and for which we have more data to base our conclusions on. First the correlation between EMG recorded from a muscle and the torque generated by that muscle. Although the correlation is not perfect, EMG can provide a reasonable estimate of torque. Second, we examine the amount of extension fatigue produced by long (4-5 minutes), continuous stimulation. Both large and small response fatigue could be recorded at four minutes, but it was difficult to correlate with specific sites in the spinal cord. (2) We continued our studies examining sites in the sacral spinal cord which produce penile erection to microstimulation of the spinal cord during this quarter. A manuscript summarizing that data has been submitted for publication. Some of those results not present in previous progress reports will be presented now. (3) Tracing studies using pseudorabies virus (PRV) to determine the location and distribution of efferent neurons and interneurons which control ejaculation continued during this quarter. In these studies, the prostate gland was injected with tracers and labeled neurons were found in both the sacral (parasympathetic) and lumbar (sympathetic) spinal cord.

II. Hindlimb Flexion and Extension Torque and EMG Produced by Microstimulation of the Spinal Cord

The correlation of the magnitude of the EMG with magnitude of torque or force generated by a muscle has been somewhat controversial in the literature. In these studies, we examined the

relation of EMG to torque generated by the extensors and flexors of the cat hindlimb. These studies were part of our mapping experiments to determine the reliability of EMG as a predictor of torque and as an indicator of selectivity.

The methods used in these studies have been described in previous progress reports. Cats were anesthetized with pentobarbital (25-35mg/kg iv). A rotational torque sensor positioned over the knee joint was attached to the shank by a metal bar. EMG electrodes were placed in the quadriceps and hamstrings (biceps femoris and semitendinous). Fine tipped iridium microelectrodes were used to stimulate sites at 200μm intervals. The torque generated at each site to the standard stimulus (100μA, 0.2 ms peak duration at 40 Hz) was averaged over the first 12 seconds of a 30 second stimulus. Likewise, the rectified raw EMG was integrated over the first 12 seconds. This data for 163 locations along seven electrode tracks in L_6 is plotted in Figure 1. The left graph plots flexion or extension EMG as a function of extension torque and the right graph as a function of flexor torque. The best fit linear regression is shown for each extension or flexion. These graphs show that when extension torque was large extension EMG was also larger and flexion EMG small. Likewise, when flexion torque was large, flexion EMG was also large and the extension EMG smaller. The differences seen with flexion torque are, however, much smaller. In general, the flexor muscles are smaller and weaker than extensor muscles. This is especially true in the L_6 segment which is more effective in generating extension torque than flexion torque.

Although EMG levels are not a perfect predictor of torque, it can indicate the relative degree of co-contraction of two groups of muscles.

During this quarter we also examined the muscle fatigue that is produced by long

continuous activation of a muscle group. Muscle fatigue has always been a concern of investigators developing motor prosthetic devices. In these studies we examined the extension fatigue that occurred with a standard stimulus (100μA, 40 Hz, 0.2 ms pulse duration) delivered for four minutes instead of the usual 30 second stimulus application. Figure 2 shows examples of two quite different sites. One shows rapid fatigue while the other actually shows an increase in torque with time. Variation in fatigue was seen throughout the ventral horn. No location could be ascribed to a particular pattern of fatigue production.

These types of studies will continue in the next quarter with the use of small electrode arrays to further examine the muscle fatigue produced by spinal cord microstimulation.

III. Penile Erection Produced by Microstimulation of Sacral Spinal Cord

The purpose of these studies was to determine sites in the lumbosacral spinal cord which produce increases in cavernous sinus pressure to focal microstimulation with fine tipped microelectrodes. Change in cavernous sinus pressure is used in these studies as a quantitative measure of penile erection. In our initial experiments in cats, we have shown that the same stimulus sites which produce large to moderate changes in cavernous sinus pressure produce penile elongation and tumescence. Similar responses have been reported in other animal studies as well. In the cat both the cavernous sinus pressure response and penile tumescence/elongation were dependant on the anesthetic used. The responses were best seen in pentobarbital anesthetized cats whereas the use of α-chloralose or halothane inhibited these responses, whether elicited by stimulation of the spinal cord, ventral roots, or pelvic nerves. These experiments were performed in our standard spinal cord preparation. Cavernous sinus pressure (CSP) was recorded

with a cannula placed in the cavernous space and secured with a suture. Bladder pressure was also recorded in these experiments using a transurethral catheter. In each experiment the sacral ventral roots were stimulated to determine the spinal segment which produced the largest CSP response. This was usually the S_1 segment. Table I summarizes the CSP and bladder response elicited by ventral root stimulation from 11 animals used in these experiments. Not shown in Table I but observed during the experiments are the somatic responses (leg, foot, and ankle extension and flexion) also elicited by ventral root stimulation. These nonspecific or unwanted responses were often seen with ventral root stimulation. Once the spinal segment which produced the best response was determined, that segment was mapped for changes in CSP. Table 2 summarizes the maximal CSP responses elicited by microstimulation of the spinal cord in each experiment. The change in bladder pressure produced by stimulation at that same site is also shown. With microstimulation of the spinal cord, the large somatic responses seen with root stimulation are greatly reduced or eliminated. Figure 3 summarizes the location of sites in S_1 spinal cord which produces increases in CSP. Sites are in a long thin brand extending from the sacral parasympathetic nucleus to the base of the ventral horn. These stimulus sites overlay with neuroanatomical sites which include parasympathetic preganglionic neurons and their axons, motoneurons which likely innervate striated muscle of the bulbocavernous and ishocavernous muscle both of which are involved in erectile reflexes. This area also contains interneurons which are visualized with the neuroanatomical tracer, pseudorabies, when injected into the penis. The results of this study indicate that penile erection can be elicited by microstimulation of a rather small group of neurons in the caudal to middle S_1 spinal cord. These responses should be enhanced and the current density reduced by stimulation with a small array of electrodes with an

orientation in the rostrocaudal and possibly the dorsoventral directions. Experiments using small arrays of electrodes will begin in the next quarter.

IV. Pseudorabies Virus Tracing Experiment to Determine the Location of Neurons Involved in Ejaculation

The purpose of this study was to determine the location of neurons which are involved in ejaculatory responses in male cats. These locations would provide targets for further studies using microstimulation technique to produce ejaculation.

Pseudorabies virus (PRV) was injected bilaterally into the prostate gland at the level of the entrance of the vas deferens. PRV labeled neurons were found at both the sacral (S_1, S_2, and S_3) and lumbar (L_2, L_3, and L_4) levels of the spinal cord corresponding to the parasympathetic and sympathetic innervation of the prostate and vas deferens. At the sacral level, the S_2 segment had densest PRV labeling (Figure 4). Labeled neurons were seen in the sacral parasympathetic nucleus (SPN), the dorsal commissure (DCM), and the superficial layers of the dorsal horn. Some of the neurons on the SPN appeared to be preganglionic efferent neurons, but many small interneurons were also labeled in the SPN. The DCM also contained many labeled interneurons. Neurons labeled in DCM have been common finding for organs innervated by the sacral parasympathetic outflows. DCM has been labeled following bladder, penile, and sphincter injections of PRV.

Labeled neurons were also seen in the lumbar segments of the spinal cord especially in the L_3 segment. Here the intermediolateral cell columns (IML) were densely labeled in a small

circumscribed area of the IML (Figure 4). The majority of these neurons were probably preganglionic sympathetic neurons which innervate the prostate and vas deferens and provide its excitatory input. Labeled neurons were also seen extending from the IML toward the central canal and the DCM (Figure 4). These were probably interneurons although preganglionic neurons displaced toward the central canal have been reported at lumbar levels of the spinal cord.

Since the sympathetic neurons are thought to be the major excitatory input to the prostate and vas deferens, this would be an important target for microstimulation. The parasympathetic neurons at the sacral levels are thought to be excitatory to the secretory glands of the prostate. The possibility that some of the sacral cord label is due to urethral innervation cannot be completely discounted since nerve fibers to the underlying urethra probably pass through and over the surface of the prostate gland.

These studies will continue into the next quarter and will expand to include the striated muscle tissue involved in the ejaculatory response (i.e., bulbocavernous and ishocavernous muscles).

Table 1: Summary of ventral root stimulation. Stimulation: 0.05ms pulsewidth, 0.5V-10V intensity and 15Hz-35Hz frequency. X - no data.

Cat #	Max. CSP (cmH_2O)			Max. Bladder Pressure (cmH_2O)		
	S1	S2	S3	S1	S2	S3
1	135	75	0	X	X	X
2	125	0	0	25	45	0
3	30	25	0	0	35	0
4	75	0	0	0	12	0
5	25	0	0	0	60	0
6	0	25	X	0	65	X
7	75	10	X	10	50	X
8	75	20	X	0	30	X
9	150	0	X	0	25	X
10	25	0	0	25	0	50
11	110	62	0	0	30	0
Mean±SE	75±15	20±8	0±0	6±3	35±6	8±4

Table 2: Summary of microstimulation in spinal cord. Stimulation: 0.2ms pulsewidth, 25Hz-35Hz frequency and 50μA-100μA. X - no CSP response.

Cat #	Max. CSP (cmH_2O)	Bladder Pressure (cmH_2O)	Depth in cord (mm)	Cord segment
2	100	5	1.8	S1
3	X			S1
6	100	12	1.6	S2
8	90	10	2.0	S1
9	125	10	1.8	S1
10	100	15	2.2	S1
11	60	15	2.6	S1
Mean±SE	96±9	11±4	2.0±0.2	

Figure 1 Relation between the EMG and the isometric extension and flexion torque in all mapped locations of the L_6 spinal cord for the same animal. The thick line is the linear regression of the extensor data and the thin line is the linear regression of the flexion data. Note the horizontal and the vertical axis's scale difference between the left and right graphs.

Figure 2 Fatigue variation with microstimulation locations. Stimulation: 0.2 ms, 40 Hz and 100 μA. A: microelectrode at a depth of 4.2 mm from the surface of the L_6 spinal cord; B: the same depth as A, but 500 μA caudal to A.

Figure 3 Effective microstimulation sites which produced change of CSP greater than 90 cmH_2O (•), 60 cmH_2O (◦) and 30 cmH_2O (+) respectively.

Figure 4 Camera lucida drawings of transverse sections of the lumbar (L_2, L_3, and L_4) and sacral (S_1, S_2, and S_3) spinal cord showing the location and distribution of PRV labeled neurons. Neurons from two sections are superimposed for each drawing. Bar = 600μ.

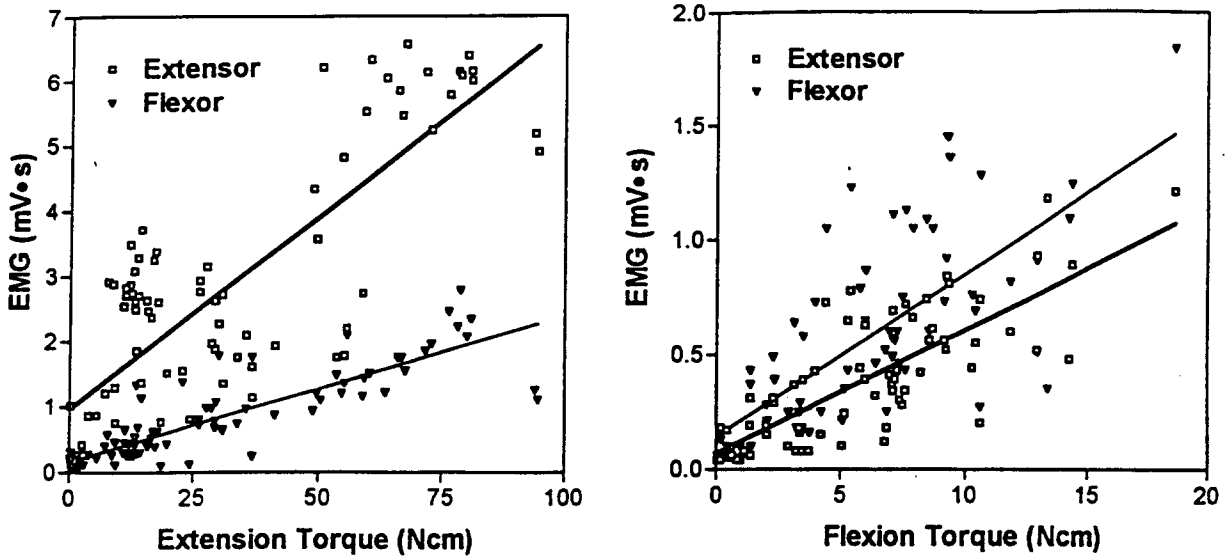


Figure 1

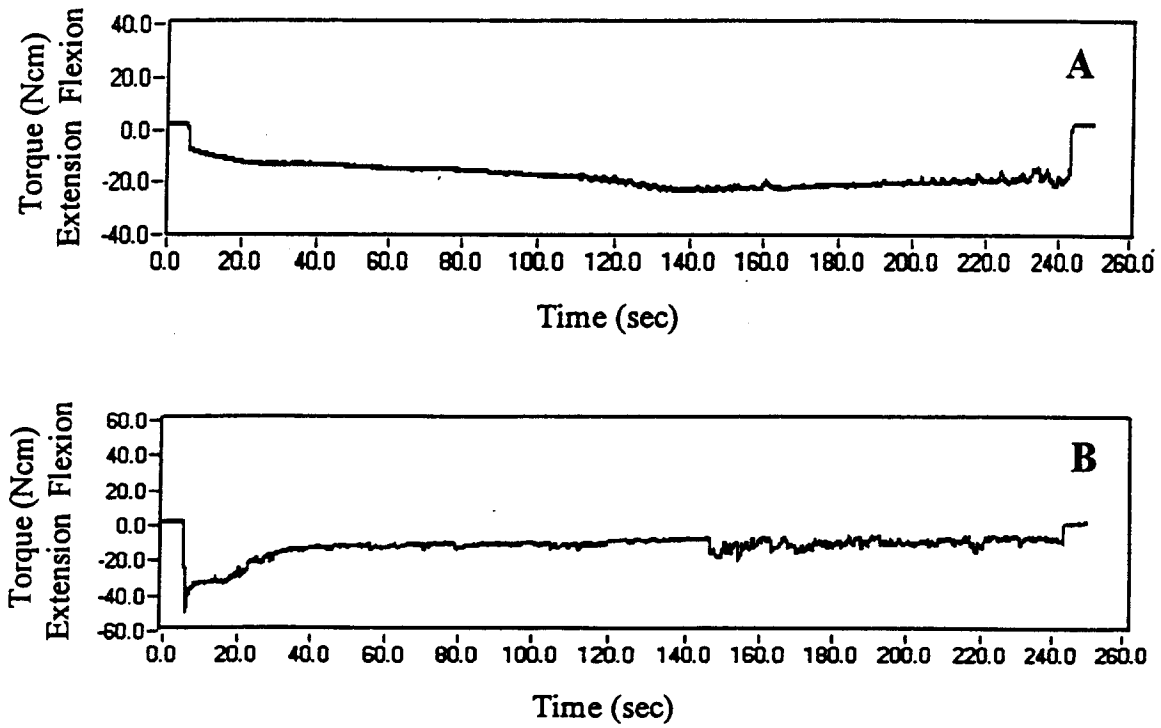


Figure 2

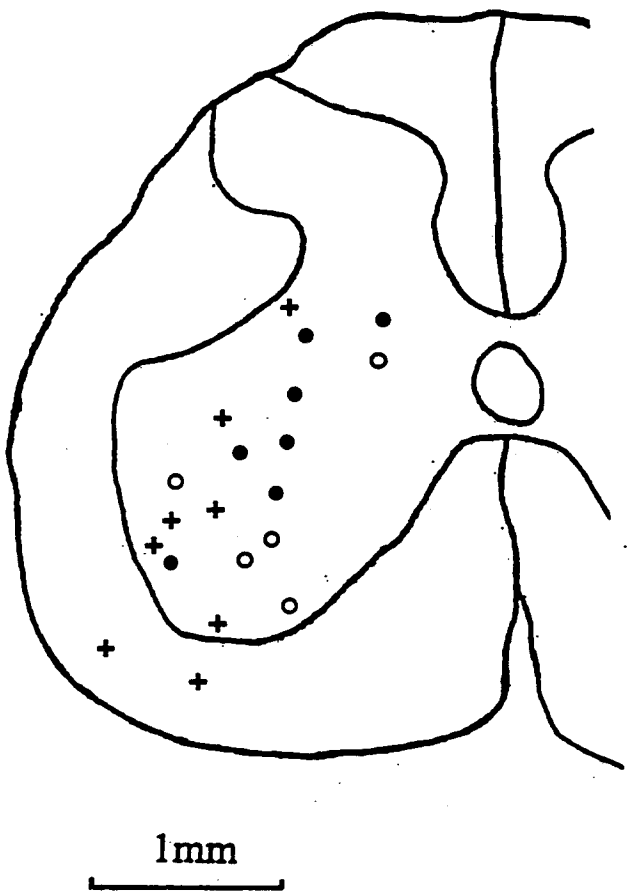
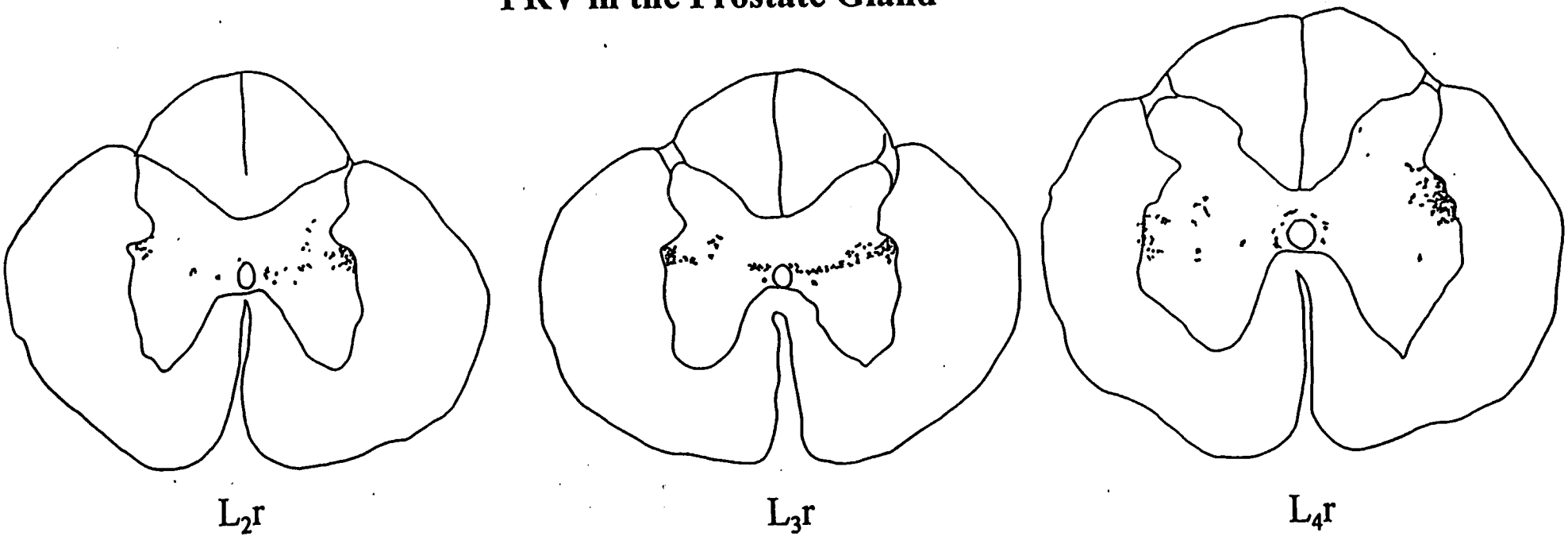
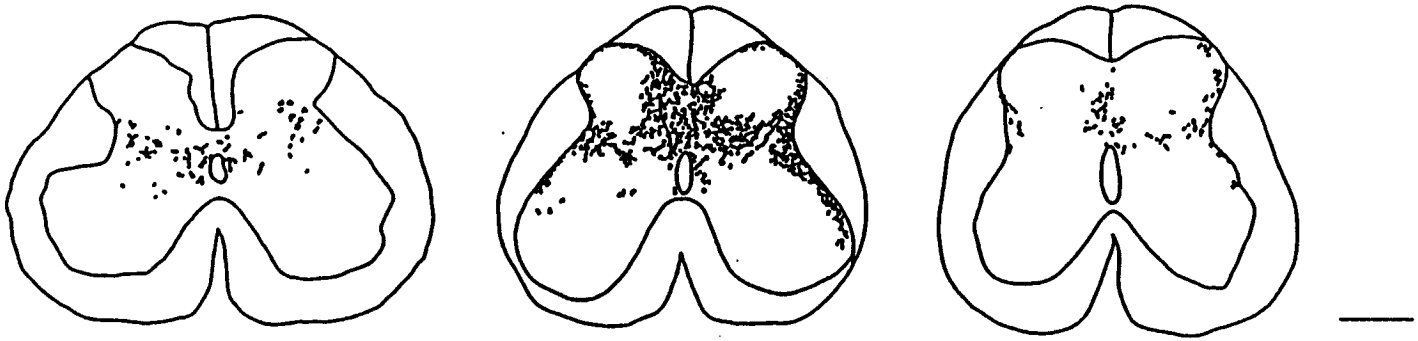


Figure 3

PRV in the Prostate Gland



L_2r L_3r L_4r



S_1r S_2r S_3r

Figure 4